XXXIX. THE METHYLATION OF HEXOSEMONOPHOSPHORIC ESTER.

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IN the original description of hexosemonophosphoric ester prepared by the action of yeast juice on fructose and glucose Robison [1922] expressed the opinion that the compound was probably a mixture of glucose- and fructose-monophosphoric esters. This conclusion was based on the properties of the dextrorotatory sugar obtained by acid hydrolysis of the ester and on the positive Selivanoff reaction for fructose given by all samples of the purified hexosemonophosphoric acid.

Meyerhof and Lohmann [1927] obtained further experimental evidence that the hexosemonophosphoric acid described by Robison was a mixture of an aldose- and a ketose-phosphoric ester. By estimating the reducing power according to Bertrand's method and comparing this figure with that obtained when using the Willstätter and Schudel [1918] hypiodite method they were able to calculate the percentage of aldosemonophosphoric ester present in the total hexosemonophosphoric acid. Lohmann [1928] attempted to separate these aldose and ketose constituents of hexosemonophosphoric ester by acid hydrolysis, since in the event of the ketosemonophosphoric acid present being identical with the fructosemonophosphoric acid prepared by Neuberg [1918] it should be more easily hydrolysed than the remaining aldosemonophosphoric acid. After partial hydrolysis Lohmann obtained an ester which showed an enhanced aldose content together with a specific rotation $[\alpha]_D^{18} + 35.7^\circ$ for the free acid. He did not succeed however in completely eliminating the ketose component from the aldosemonophosphate.

Robison and King [1929] by fractional crystallisation of the brucine salt of hexosemonophosphoric ester obtained a compound possessing a higher dextrorotation ($[\alpha]_{5461}^{20} + 21^\circ$ for the barium salt) than the original ester and an increased content of aldosemonophosphate (91 %) as measured by the iodimetric method modified according to Macleod and Robison [1929].

Towards the end of 1928 Robison and King had separated the aldosemonophosphate from yeast hexosemonophosphate and had obtained strong evidence that it was a monophosphoric ester of glucose. Their experiments yielded

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sufficient of this purified aldosemonophosphate to enable work to be commenced on its methylation with a view to proving its structure.

The results of the methylation of this hexosemonophosphate were given in a short abstract by King and Morgan [1929, 1]. It was shown that if this hexosemonophosphate was methylated by Fischer's method, both at room temperature and at 60°, two distinct hexosides were formed. The hexoside produced at room temperature was rapidly hydrolysed by 0.1 *N* acid at 35°, the methyl-group being completely eliminated in about 24 hours. The hexoside produced by methylation at the higher temperature was not hydrolysed under these conditions. These two hexosides probably represent the reactive (furanose) and the stable (pyranose) forms whose formation could only take place if the hydroxyl-groups attached to the carbon atoms 4 and 5 are unsubstituted. It appeared therefore that the phosphate group was not in either of these positions. The earlier work of Robison [1922] had eliminated the possibility of the phosphoric acid group being attached in positions 1 and 2. There remained however positions 3 and 6. King and Morgan pointed out that while no definite conclusions could be reached as to the exact position of the phosphoric acid group, the rate of hydrolysis of this group pointed to position 6 as the most probable (I) but this inference appeared to be incompatible with the difference in the melting points of the osazones of hexosemonophosphoric acid and hexosediphosphoric acid which had been reported by Robison. It was therefore suggested [King and Morgan, 1929, 2] that the phosphoric acid group was in position 3 (II). Levene and Raymond [1929] having observed the formation of a reactive γ -glucoside when hexosemonophosphoric acid was methylated by Fischer's method at room temperature, concluded that this

phosphoric ester possessed an unstable furanose ring, the phosphoric acid group being attached to the fifth carbon atom (III). Methylation at these temperatures is, however, considered to favour the production of a γ -hexoside and, therefore, cannot be used as evidence that the phosphoric acid group is in the fifth position and has thus prevented the formation of the normal 1:5 ring.

Josephson and Proffe [1930] converted di-isopropylideneglucose by the action of phosphoryl chloride in pyridine solution at -18° to -20° into a di-isopropylideneglucose phosphate which on treatment with baryta and subsequent hydrolysis yielded a glucose-3-phosphate possessing a pyranose ring

structure. These authors claim that Robison's ester is not identical with their synthetic ester and, therefore, cannot be a glucose-3-phosphoric acid as suggested by King and Morgan.

The methylation experiments quoted in the original abstract of King and Morgan have been repeated using further preparations of purified aldosemonophosphate and the production of the two different types of hexosides has been confirmed. The evidence as to the nature of the oxide ring system in these two different types of hexosides has been further developed by comparing the rate of hydrolysis of the dephosphorylated methylhexosides with a methylhexoside of known structure. In the absence of any evidence which points to the migration of the phosphoric acid group during methylation it would appear highly probable that positions 4 and 5 in aldosemonophosphoric acid are not substituted by the phosphoric acid group. Meanwhile Robison and King [1929, 1931] isolated the pure aldosemonophosphoric acid component of hexosemonophosphoric acid and the determination of the melting point of its osazone showed this to be identical with that of the osazone of fructosemonophosphoric acid derived from fructosediphosphoric acid in which the phosphoric acid group is most probably in position 6 [Morgan and Robison, 1928; Morgan, 1929]. From this evidence the aldosemonophosphoric acid would appear to be an aldose-6-phosphoric acid and this conclusion is to some extent supported by the recent work of Levene and Raymond [1930]. These authors have also prepared the synthetic glucose-3-phosphoric acid and have shown that it differs markedly in properties from the Robison hexosemonophosphate. They have also prepared another synthetic ester which they consider to be glucose-6-phosphoric acid and which resembles the Robison ester in some though not in all respects [Robison and King, 1931].

EXPERIMENTAL.

The hexosemonophosphoric ester used in the methylation experiments was prepared by the fractional crystallisation from 20 % ethyl alcohol of the brucine salt of the crude ester isolated from the products of yeast juice fermentation, and a typical analysis of the purified barium hexosemonophosphate is given below.

Total phosphorus	...	...	7.6 %
Inorganic phosphate	...	...	Absent
Reducing power (H. and J.)	...	...	35.4 %
Iodine reduction (M. and R.)	...	...	39.3 % ($\equiv$ 87 % aldose component)
$[\alpha]_{5461}^{18^\circ}$	...	...	+ 20.4°

METHYLATION OF THE HEXOSEMONOPHOSPHATE.

Methylation at 18°. Barium hexosemonophosphate (9.2 g.) was added to 200 cc. of anhydrous methyl alcohol containing hydrogen chloride in amount

calculated to give a final solution containing 0.5 % of free hydrogen chloride. To facilitate the rapid solution of the salt the mixture was shaken under conditions previously described in detail by Morgan [1927]. The barium salt dissolved rapidly to give a clear pale yellow solution and no separation of barium chloride occurred during the esterification. The progress of the esterification was followed by estimating the reducing power of the solution by the method of Hagedorn and Jensen and by frequent readings of the rotation in a 4 dm. polarimeter tube. During the first hour there was a sharp rise in the observed rotation followed by a sudden drop, the rotation continuing to decrease until esterification was complete. The course of this change is shown in Fig. 1, Curve A, while the analytical figures obtained during the esterifica-

Fig. 1. Change in optical rotation during methylation of hexosemonophosphoric acid.

A. At 18°.

B. At 60°.

Table I.

Time		Observed rotation [α] ₄₄₆₁	Total phosphorus g.	Reducing power as glucose per 1 cc. of the solution mg.	Esterifi- cation %
Hrs.	Mins.				
	5	4.67°	0.692	14.8	0
	10	4.74	—	—	—
	25	4.77	—	—	—
	40	4.79	—	—	—
	55	4.81	—	—	—
1	25	4.67	—	—	—
2	10	4.49	—	—	—
3	30	4.36	—	—	—
7	25	3.79	0.691	11.70	21
18		2.72	0.694	9.40	36
24		2.72	—	—	—
42		1.43	0.685	6.45	56
67		1.03	0.682	5.35	64
93		0.88	0.687	3.95	73
115		0.82	0.689	3.05	79
160		0.79	0.690	2.70	85
211		0.63	—	1.32	91
*260		—	—	0.12	99

* After re-solution in fresh methyl alcoholic hydrogen chloride.

tion are given in Table I. There was no formation of inorganic phosphate during the experiment. The reduction at the end of 8 days indicated that 90 % of the theoretical quantity of hexoside had been produced. The yield of methylhexosidemonophosphate was, however, considerably increased by

neutralising the 90 % equilibrium mixture, distilling off the methyl alcohol under reduced pressure at 35°–40°, and redissolving the hexose-hexoside mixture obtained in 200 cc. of dry methyl alcohol containing hydrogen chloride as described above. Under these conditions the solution became almost non-reducing (less than 1 %) and the methylhexosidemonophosphoric ester was obtained in an almost quantitative yield. The acid methyl alcohol solution of the hexoside was neutralised by the addition of silver carbonate, the precipitated silver chloride filtered off and the filtrate made just pink to phenolphthalein by the addition of methyl alcoholic solution of baryta. The precipitated barium methylhexosidemonophosphate was washed with anhydrous methyl alcohol and dried in a vacuum desiccator. The crude barium salt thus obtained was dissolved in 10 parts of water and fractionally precipitated with increasing concentrations of ethyl alcohol. The following fractions were collected:

Alcohol concentration	Weight of fraction
0–30 % (by volume)	3.3 g.
30–40 % "	2.1 g.
45–80 % "	4.5 g.

The optical rotation of these fractions showed no appreciable difference, thus indicating no separation into the α - and β -methylhexosidemonophosphates. That the α - and β -isomers were present seemed certain from the type of the methylation curve (Fig. 1, Curve A), the preliminary rise being due to the formation of a hexoside possessing greater dextrorotation than the original mixture of α - and β -hexosemonophosphoric acids. In the same way the subsequent fall in rotation presumably indicated the formation of the more laevorotatory isomer.

In another methylation under similar conditions 6.1 g. of barium hexosemonophosphate were dissolved in 300 cc. of anhydrous methyl alcohol containing hydrogen chloride. The reaction was stopped by the addition of excess barium carbonate when 93 % esterification had been reached. The solution was filtered and a hot saturated aqueous solution of baryta added to the filtrate until it was just pink to phenolphthalein. Two volumes of light petroleum and half a volume of ether were added, the resulting precipitate was collected by centrifuging and dried in a vacuum desiccator. The crude barium salt thus obtained, containing barium chloride as the principal impurity, was dissolved in 8 times its weight of water. The barium chloride was precipitated by the addition of a saturated aqueous solution of silver sulphate, the end-point being determined by centrifuging a small sample of the liquid and testing with silver sulphate solution. A slight trace of barium chloride amounting to, at most, 0.15 % of the total solid was left in the solution. The resulting precipitate was centrifuged off and washed with water. The large volume of centrifugate was reduced *in vacuo* over concentrated sulphuric acid to approximately the original volume, when a dark-coloured solution was obtained. To this solution slightly more than sufficient normal sulphuric acid

to give the free hexoside was added. The barium sulphate was quickly centrifuged off, the remaining trace of silver chloride being also removed. The clear colourless solution was then made pink to phenolphthalein by the addition of a cold saturated solution of baryta. The solution was then evaporated to the original volume and centrifuged to remove barium sulphate resulting from the slight excess of sulphuric acid. It was then treated with a mixture of alcohol, ether and light petroleum. The resulting precipitate, after drying over sulphuric acid *in vacuo*, gave the following analytical figures:

	% OCH ₃	% P	% Ba
Found	7.57	7.45	32.98
Calculated for C ₈ H ₁₀ O ₄ (OCH ₃)(PO ₄ Ba)	7.57	7.57	33.55

Methylation at 60°. Barium hexosemonophosphate (6.0 g.) was added to 100 cc. of anhydrous methyl alcohol containing hydrogen chloride. The general method and precautions observed were the same as those described above. A portion of the methyl alcoholic solution was at once transferred to a jacketed 4 dm. polarimeter tube which was kept, together with the remaining part of the solution, at 60° in an electric oven. At the end of the first hour barium chloride began to crystallise from the alcoholic solution, thus making it necessary to filter the solution before the polarimeter readings could be taken. The course of the methylation was followed and the resulting methylhexosemonophosphate was isolated as described for the first methylation at 18°. The figures obtained are given in Table II. No inorganic phosphate was

Table II.

Time		Observed rotation [α] _{D461}	Total phosphorus g.	Reducing power as glucose per 1 cc. of the solution mg.	Esterification %
Hrs.	Mins.				
	12	5.22°	—	15.2	—
	40	4.79	0.430	10.8	29
1	20	2.62	—	—	—
18	30	2.20	—	5.0	67
20		2.15	—	—	—
26		2.71	—	—	—
41		3.25	0.410	2.3	85
66		4.35	—	1.1	93
99		5.25	—	—	—
101		5.45	—	—	—
103		5.62	0.408	0.6	96
120		5.75	0.410	—	—

liberated during the methylation. It will be seen that the curve showing change in rotation during methylation (Fig. 1, Curve B), is entirely different in form from the corresponding curve for the methylation at 18°. The curve shows a rapid fall during the first 20 hours, followed by a steady rise until the esterification is complete, the final value for the observed rotation being higher than the original value for the hexosemonophosphoric acid. In a second methylation at 60° 6.1 g. of barium hexosemonophosphate were dissolved in 300 cc. of anhydrous methyl alcohol containing hydrogen chloride. The separation of the hexoside and its purification by means of silver sulphate were carried out

in the manner already described under the methylation at 18°. On analysis the methoxyl values were found to be higher than that calculated for barium methylhexosidemonophosphate. It was thought that this might possibly be due to partial methylation of the phosphate group, although potassium dihydrogen phosphate under the same conditions showed no evidence of esterification, a result in agreement with that obtained by Morgan [1927]. The brucine salt was prepared and was repeatedly recrystallised from aqueous alcohol and thereafter reconverted into the barium salt. The methoxyl content of the latter, though somewhat lower than before, was still higher than the calculated figure. Under the conditions employed it is possible that the dimethylacetal derivative would also be formed and might account for the high methoxyl value obtained. The regenerated barium salt gave the following analytical results:

	% OCH ₃	% P	% Ba
Found	8.23	7.49	32.76
Calculated for C ₈ H ₁₀ O ₄ (OCH ₃)(PO ₄ Ba)	7.57	7.57	33.55

ACID HYDROLYSIS OF THE METHYLHEXOSIDEMONOPHOSPHATES.

Hexosidemonophosphate formed at 18°. The barium methylhexosidemonophosphate (47.4 mg.) was dissolved in 10 cc. of 0.1 *N* HCl for the determination of the rate of elimination of the methyl group at 37°. The extent of hydrolysis was measured from time to time by estimating the reducing power of the solution by the Hagedorn and Jensen method. The results shown in Fig. 2,

Fig. 2. Hydrolysis in 0.1 *N* HCl at 37°.

- A. Methylhexosidemonophosphate from methylation at 18°.
- B. Methylhexosidemonophosphate from methylation at 60°.
- C. Dephosphorylated methylhexosidemonophosphate from methylation at 18°.
- D. Dephosphorylated methylhexosidemonophosphate from methylation at 60°.
- E. α -Methylglucoside.

→ At these points the solutions were made *N* with HCl and heated at 100° for the times indicated.

Curve A, indicate that hydrolysis was almost complete at the end of 24 hours. This was confirmed by raising the concentration of HCl to *N* and heating at

100°, when the reducing power, expressed as % of the barium methylhexoside originally present, was increased only from 37.2 to 40.9.

Hexosidemonophosphate formed at 60°. The barium methylhexosidemonophosphate (51.1 mg.) was dissolved in 10 cc. of 0.1N HCl and kept at 37°, the rate of removal of the methyl group being measured as in the previous acid hydrolysis. Curve B, Fig. 2 shows that a small but rapid rise in the reducing power occurred during the first few hours; this was possibly due to the presence of the dimethylacetal derivative mentioned above together with any of the more reactive hexosides which may have been produced during the methylation. No further increase was observed after 24 hours and the reducing power at this point indicated that only 22 % of the total hexoside had been hydrolysed. This amount presumably includes the dimethylacetal derivative, which by calculation from the methoxyl content may have been present to the extent of 20 %. At the end of 50 hours the methylhexoside solution was made *N* with hydrochloric acid and heated at 100°, and after this treatment the reducing power was found to be very nearly equal to that calculated assuming complete hydrolysis of the hexoside.

These experiments indicate that the hexoside produced by methylation at 60° is not hydrolysed to any appreciable extent under conditions which bring about almost complete hydrolysis of the methylhexosidemonophosphate produced at 18°.

THE ENZYMIC DEPHOSPHORYLATION OF THE METHYLHEXOSIDEMONOPHOSPHATES.

Methylhexosidemonophosphate formed at 18°. A sample of the purified barium methylhexosidemonophosphate (0.503 g.) was dissolved in water and the equivalent amount of potassium sulphate added. The barium sulphate was removed by centrifuging and the solution of the potassium salt adjusted to p_H 8.4 with a dilute solution of potassium hydroxide. The bone phosphatase preparation, 100 mg. [Martland and Robison, 1929], was then added together with a few drops of chloroform, the p_H again adjusted to 8.4 and the enzymic hydrolysis allowed to proceed at room temperature. Estimations of inorganic phosphate showed that 91 % of the phosphorus was eliminated at the end of 72 hours and that no further hydrolysis occurred in a further 24 hours. At this stage the solution was treated with four times its volume of alcohol, and after filtration the alcoholic solution was evaporated to dryness. The residue was extracted with boiling ethyl alcohol and the alcoholic extract filtered and evaporated. The resulting syrup was then extracted with a small quantity of water, filtered and again evaporated to dryness, when it yielded 210 mg. of a syrup which was free from phosphorus and possessed a reducing power of only 4.5 %. After drying over phosphorus pentoxide *in vacuo* the methoxyl was determined:

Found	14.67 %
Calculated for $C_6H_{11}O_8(OCH_3)$	15.98 %

Methylhexosidemonophosphate formed at 60°. The purified barium methylhexosidemonophosphate (0.638 g.) was converted to the potassium salt and treated with 500 mg.¹ of the bone phosphatase preparation under conditions similar to those described above. At the end of 24 hours 93.5 % of the phosphorus was eliminated and no further hydrolysis took place during the next 24 hours. The dephosphorylated hexoside was then isolated and purified as described above. Analysis for methoxyl gave the following results:

Found	12.73 %
Calculated for $C_6H_{11}O_6(OCH_3)$	15.98 %

The methoxyl value indicates that the product contained a considerable proportion of contaminating material which possibly may have been derived from the phosphatase preparation of which an unnecessarily large amount was used for the hydrolysis.

THE ACID HYDROLYSIS OF THE DEPHOSPHORYLATED METHYLHEXOSIDEMONOPHOSPHATES.

A carefully dried sample of the dephosphorylated methylhexosidemonophosphate (0.114 g.) produced by methylation at 18° was dissolved in 40 cc. of 0.1 *N* HCl and kept at 37°. The extent of the removal of the methyl group was measured by estimating the reducing power of the solution by Hagedorn and Jensen's method as in the acid hydrolysis of the methylhexosidemonophosphate. The optical rotation of the solution in a 4 dm. tube was observed during the hydrolysis. The results are shown in Fig. 2, Curve C; Fig. 3, Curve A;

Fig. 3. Change in optical rotation during hydrolysis in 0.1 *N* at 37°.

A. Dephosphorylated methylhexosidemonophosphate from methylation at 18°.

B. Dephosphorylated methylhexosidemonophosphate from methylation at 60°.

→ At these points the solutions were made *N* with HCl and heated at 100° for 1 hour.

and in Table III, and indicate the extreme ease with which the methyl group is eliminated under these conditions. About 80 % hydrolysis was effected in 12 hours and this figure was not increased in the following 12 hours. Heating at 100° for 1 hour in *N* HCl increased the hydrolysis to 87 %. At the end

¹ This amount was used in error, in place of 100 mg.

Table III.

Time		% hydrolysis (H. and J.)	Observed rotation [α] ₅₄₆₁
Hrs.	Mins.		
0	0	4.87	-0.21°
*0	30	21.29	-0.06
1		27.30	-0.04
2	52	51.70	+0.09
5	44	67.77	+0.30
12		81.32	+0.43
22	15	81.81	+0.47
†23	15	87.72	+0.36

* 39 cc. of the original solution + 0.4 cc. 10 *N* HCl + 0.6 cc. H₂O.

† After heating 30 cc. of the above solution + 8 cc. 10 *N* HCl + 2 cc. H₂O at 100° for 1 hour.

of the hydrolysis at 37° the specific rotation, based on the weight of material taken was [α]₅₄₆₁ + 44.0°, and on the glucose present as indicated by Hagedorn and Jensen's method was [α]₅₄₆₁ + 55.6°. After hydrolysis at 37° the reduction given by the iodimetric method of Macleod and Robison was 77.7 %.

Table IV.

Time		% hydrolysis (H. and J.)	Observed rotation [α] ₅₄₆₁
Hrs.	Mins.		
0	0	1.8	+0.30°
*0	30	4.5	+0.27
1	15	7.7	+0.27
3	10	12.4	+0.32
6	15	14.2	+0.36
11	40	17.1	+0.38
23		20.5	+0.395
†23	30	66.5	+0.40
†24		67.6	+0.36

* 36.5 cc. of the original solution + 0.4 cc. 10 *N* HCl + 3.1 cc. H₂O.

† After heating 35.2 cc. of the above solution + 4 cc. 10 *N* HCl + 0.8 cc. H₂O at 100° for 30 minutes.

‡ 30 minutes' further heating at 100°.

A sample of the dephosphorylated methylhexoside (0.116 g.) obtained from the product of methylation at 60° was dissolved in water, made up to 40 cc. and the reducing power and rotation of the solution were determined. The course of the hydrolysis was followed by frequent estimations of the reducing power (Hagedorn and Jensen) of the solution and by the change in optical rotation as observed in a 4 dm. tube. Fig. 2, Curve D shows that at the commencement of the hydrolysis there was a rapid but limited rise in the reducing power of the solution indicating the presence of about 20 % of a substance readily hydrolysed by 0.1 *N* HCl at 37°. After 20 hours, as no further hydrolysis appeared to take place, the solution was made *N* with hydrochloric acid and heated at 100° for half an hour. This procedure raised the percentage hydrolysis from 20.5 to 66.5, while after a further 30 minutes at 100° this figure was only increased to 67.6. The iodine reduction value determined at this stage corresponded with 74.9 % hydrolysis. The specific rotation after 30 minutes' heating at 100° calculated on the basis of the glucose present (Hagedorn and Jensen) was [α]₅₄₆₁²⁰ + 69.6° and after a further period of

30 minutes at 100° , $+61.5^{\circ}$. The change in optical rotation of the solution during hydrolysis is shown in Fig. 3, Curve B.

The dephosphorylated methylhexosides derived by enzymic hydrolysis from the products of methylation of aldosemonophosphate at 18° and 60° respectively exhibit the same pronounced difference in their rates of hydrolysis by $0.1N$ HCl at 37° as do the methylhexosidemonophosphates themselves. It is clear from the curves that the presence of the phosphate group does not materially affect the rate of hydrolysis of the methoxyl group.

In order to compare the rates of hydrolysis of these hexosides with that of a compound of known constitution a sample of recrystallised α -methylglucoside was hydrolysed by $0.1N$ HCl at 37° followed by N HCl at 100° . The results are shown in Fig. 2, Curve E. The reducing power and the specific rotation of the hydrolysis product were determined in a separate experiment in which 0.1327 g. of α -methylglucoside in 50 cc. of $2N$ HCl was heated at 100° for 1 hour. Calculated from the reducing power determined by the Hagedorn and Jensen method, the hydrolysis amounted to 96.5% , and the specific rotation $[\alpha]_{5461}^{20^{\circ}}$ of the product was $+65.9^{\circ}$. The reducing power by the iodimetric method, however, indicated complete hydrolysis and on this basis the specific rotation was $+63.4^{\circ}$. These values for the specific rotation are somewhat higher than that of glucose in aqueous solution ($[\alpha]_{5461}^{20^{\circ}} + 62.5^{\circ}$), but a determination of the specific rotation of glucose in similar low concentration in $2N$ HCl gave the value $+63.6^{\circ}$, while after heating for half an hour at 100° the reducing power by the Hagedorn and Jensen method showed that some destruction of glucose had taken place (3%) and the specific rotation, calculated from this reducing power, was $+66.3^{\circ}$.

SEPARATION OF THE α - AND β -METHYLHEXOSIDEMONOPHOSPHATES.

Small amounts of the barium methylhexosidemonophosphates produced by methylation at 18° and 60° respectively were converted into the brucine salts. The barium was removed by shaking an aqueous solution of these salts with a slight excess of brucine sulphate until the reaction was complete. By allowing the alcoholic solutions of the brucine salts to evaporate slowly several crops of crystals were removed. From the brucine salt of the methylhexosidemonophosphoric ester methylated at 18° crops of crystals yielded, on converting to the barium salt, non-reducing compounds having extreme specific rotations of $[\alpha]_{5461}^{20^{\circ}} + 13^{\circ}$ and $+49^{\circ}$, while the brucine salts of the more stable hexoside yielded non-reducing barium salts possessing specific rotations of $[\alpha]_{5461}^{20^{\circ}} + 3.5^{\circ}$ and $+39^{\circ}$. These figures indicate a separation into the α - and β -isomers of the corresponding reactive and stable hexosides, but since the brucine salts were not exhaustively fractionated using different solvents it is not possible to claim that the specific rotations are those of the pure isomers.

DISCUSSION.

The simplest and most probable explanation of the experimental results described in this paper is that during the methylation of purified aldosemonophosphate two types of aldoses are formed presumably containing a reactive (furanose) or a stable (pyranose) ring structure according to the temperature of methylation. This explanation assumes that only furanose and pyranose ring structures are formed; this assumption is, however, strongly supported by a large mass of experimental work in the sugar group. At present there is no authentic case of a propylene oxide (1:3) sugar known. The structure of di-isopropylidenexylose has been investigated by Haworth and Porter [1928] who originally considered that this compound presented a unique opportunity to investigate the possible existence of a propylene oxide (1:3) ring system. They concluded, however, after careful examination, that this compound possesses a furanose (1:4) ring and thus falls into line with one of the two known oxide ring systems. It would appear, therefore, that in the absence of any evidence indicating that the two hexosides produced from the purified aldosemonophosphate by simple methylation at 18° and 60° are different from the known furanose and pyranose types, we may conclude that the phosphoric acid group is not attached at the 4th or 5th position in the aldosemonophosphate.

It may be pointed out that although the ester used in these experiments contained only 87 % of the pure aldosemonophosphate, since isolated by Robison and King [1931], the presence of the second component could not possibly account for the formation of the two methylhexosides in the yields obtained.

The recent work of Robison and King on the melting point of the osazone of the purified aldosemonophosphate and on the oxidation of this ester points to the phosphoric acid group being attached in position 6, while from the synthetic side the evidence of Josephson and Proffe seems to eliminate the possibility of the phosphoric acid being in position 3, a conclusion which is also supported by Levene and Raymond. On the evidence now available it would therefore appear probable that the aldosemonophosphate component of Robison's original hexosemonophosphate is glucose-6-phosphoric acid.

SUMMARY.

1. The methylation of the aldosemonophosphate component of Robison's fermentation hexosemonophosphate by Fischer's method at 18° and at 60° has yielded two distinct forms of methylhexosidemonophosphate.

2. The methylhexosidemonophosphate produced by methylation at 18° is extremely sensitive to acids. With 0.1 *N* acid at 36° it is rapidly hydrolysed yielding a reducing hexosemonophosphoric acid.

3. The corresponding hexoside produced by methylation at 60° is stable in the presence of 0.1 *N* acid at 37°. Its rate of hydrolysis closely resembles that of α -methylglucoside under the conditions described.

4. The bearing of these experimental results on the constitution of the aldosemonophosphate component of Robison's fermentation hexosemonophosphoric ester is discussed.

In conclusion we wish to express our gratitude to Dr R. Robison for his very helpful criticism and advice.

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